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BLOOD FLOW AND FATTY ACID RELEASE BY CERVICAL ADIPOSE TISSUE OF RABBITS

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SUMMARY

1. A direct technique was used to measure blood flow and exchange of metabolites by cervical adipose tissue in 3-month-old and adult rabbits.

2. The initial blood flow from adipose tissue of fed 3-month-old rabbits was 0·45 ml./g tissue.min and the tissue released fatty acids and glycerol. In similar rabbits unfed for 24 hr, fatty acid release increased fourfold. Both the rate of blood flow and the arterio-venous difference of fatty acid increased. In fed adult rabbits blood flow was 0·16 ml./g tissue.min.

3. Noradrenaline infusion in both young and adult rabbits stimulated a rapid and large increase in cervical adipose tissue blood flow together with an increase in fatty acid and glycerol release. Glucose uptake remained small. No increase in local heat production in cervical adipose tissue was detected during noradrenaline infusion.

4. It is concluded that cervical adipose tissue in adult rabbits is actively concerned in energy storage and supply.

INTRODUCTION

In the new-born rabbit, one form of adipose tissue, brown adipose tissue, is found in well-developed lobes in the cervical and interscapular region. The tissue is structurally distinct from the more familiar white adipose tissue, and it has the function of generating extra heat in response to cold exposure (Dawkins & Hull, 1964). At birth the tissue's stores of fatty acids are reserved for heat production (Hardman, Hey & Hull, 1969) but during the first week of life a change occurs, the thermogenic capacity of brown adipose tissue falls and the tissue begins to supply fatty acids for metabolism elsewhere (Hardman & Hull, 1970).

In the adult rabbit distinct lobes of adipose tissue are still easily recognized in the cervical region. The present investigation was designed to study the metabolic responses of cervical adipose tissue in mature rabbits.

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METHODS

The New Zealand White rabbits studied were born in the animal house and reared for the first 3 weeks of life in a thermoneutral environment and suckled by the doe once each day. They were then weaned on to a mixed diet of chow and green vegetables. Ten rabbits aged 3 months were studied, five were allowed free access to food on the day of the experiment and five were kept unfed for 24–48 hr before the experiment. Eleven adult rabbits aged 7 months were also studied in the non-fasting state.

Careful dissection of the adipose tissue in the neck revealed the same distinct lobes of tissue which are easily recognized in the new-born rabbit. Anterior and medial to the sterno-mastoid muscle is the loosely defined anterior lobe. Posterior and lateral to the sterno-mastoid is the triangular lateral lobe. This lobe is linked by vessels to the upper half of the posterior lobe which extends downwards between the muscles of the back and under the scapulae. The long interscapular lobes are more superficial, lying underneath the skin of the back on either side of the vertebral spines.

The rabbits were anaesthetized with urethane (1 g/kg body wt. I.V.). A polyethylene catheter for infusions was placed in a branch of the right external jugular vein and a second catheter inserted into the right carotid artery. A third catheter was placed in the left external jugular vein close to the junction with the left lateral vein which drains the lateral and upper half of the posterior lobes of cervical adipose tissue. By occluding the jugular vein below the entry of the lateral vein, the venous outflow from the known area of cervical adipose tissue was redirected via the catheter along a calibrated tube. This allowed the rate of flow along the tube to be measured and venous samples to be collected (Hardman & Hull, 1970).

In all the 3-month-old rabbits and in six adult rabbits, blood flow was measured and at least two arterial and venous samples of blood collected before the I.V. infusion of L-noradrenaline bitartrate (Winthrop Lab.) at the rate of 4μg base/kg. min for 10 min. Blood flow was measured and arterial and venous samples collected during and after the infusion. Each blood sample was 0·5 ml. and blood loss was replaced by an equal volume of 0·9 % sodium chloride solution.

In five of the adult rabbits the temperature of the blood flowing from the lateral vein was measured during the experiment from a copper-constantan thermocouple sited at the tip of the flow catheter. In these rabbits it was only possible to measure blood flow and take paired blood samples once before and once during the noradrenaline infusion. In all experiments colonic temperature was measured from a copper-constantan thermocouple inserted 6 cm into the rectum and recorded on a Cambridge Slow Recorder. At the end of each experiment the lateral lobe and the upper half of the posterior lobe of cervical adipose tissue, the area drained by the lateral vein, was dissected free and weighed.

The concentration of blood glucose was estimated by the method of Hugget & Nixon (1957), plasma free fatty acids by a modification of the method of Nóvak (1965) and plasma glycerol by the micro-method of Boehringer (Biochemica Test Combination). The standard deviation of the mean of replicate estimates of glucose was $\pm$ 1·0 mg/100 ml.; of free fatty acids was $\pm$ 0·035 m-equiv/l. and of glycerol was $\pm$ 0·003 m-mole/l. In three of these experiments the blood pressure was recorded from the carotid cannula using a strain gauge manometer.

RESULTS

Arterial concentrations of glucose, free fatty acids and glycerol

The initial concentrations of all three metabolites in the adult and in the fed and unfed young rabbits are given in Fig. 1. The concentrations of blood glucose were lower in the unfed rabbits, but the concentrations of free fatty acids were three times greater in unfed young rabbits than they were in fed animals. The serum glycerol concentrations were similar in all groups.

Arterial concentrations of glucose, free fatty acids and glycerol after Arven

Heparin, by its action on lipoprotein lipase, causes a marked rise in the circulating concentrations of free fatty acids and glycerol; thus it is unsuitable as an anticoagulant for these experiments. Arven, which is derived from pit-snake venom, was more satisfactory. From Fig. 1 it can be seen that administration of Arven had little or no effect on the circulating concentrations of glucose, free fatty acids or glycerol in all groups of animals.

Initial rate of blood flow and exchange of metabolites by cervical adipose tissue

The resting rate of blood flow from cervical adipose tissue of the three groups of rabbits is shown in Fig. 2 and the arterio-venous differences of glucose, free fatty acids and glycerol are given in Table 1. The initial rate of blood flow from the lateral lobe and upper half of the posterior lobe of cervical adipose tissue of the fed young rabbits was on average 0·45 ml./g tissue.min and the tissue released both fatty acids and glycerol. With starvation the resting rate of blood flow was higher and the arterio-venous difference of fatty acids was twice that of fed rabbits. In fed rabbits the net release of fatty acids was on average 0·08 μequiv/g tissue.min, whilst during starvation it increased to 0·23 μequiv/g tissue.min.

In the adult rabbits the initial rate of blood flow from cervical adipose tissue was lower than in 3-month-old rabbits. On average it was 0·16 ml./g tissue.min. The tissue released both fatty acids and glycerol, the average rate of fatty acid release being 0·03 μequiv/g tissue.min. In all the experiments the initial arterio-venous differences of glucose was small and did not differ significantly from zero.

The effect of noradrenaline on blood flow and exchange of metabolites

Fig. 2 shows the effect of noradrenaline infusion on the rate of blood flow from the cervical adipose tissue of the three groups of rabbits. The arterio-venous differences of glucose, free fatty acids and glycerol during and after the infusion are given in Table 1.

In all the rabbits noradrenaline stimulated a rapid increase in the rate of blood flow through cervical adipose tissue, which fell again when the infusion stopped. In the young fed rabbits there was also an increase in arterio-venous differences of fatty acids and glycerol. The net release of fatty acids increased from 0·08 to 0·65 μequiv/g tissue.min and glycerol from 0·05 to 0·21 μmole/g tissue.min. The increased arterio-venous

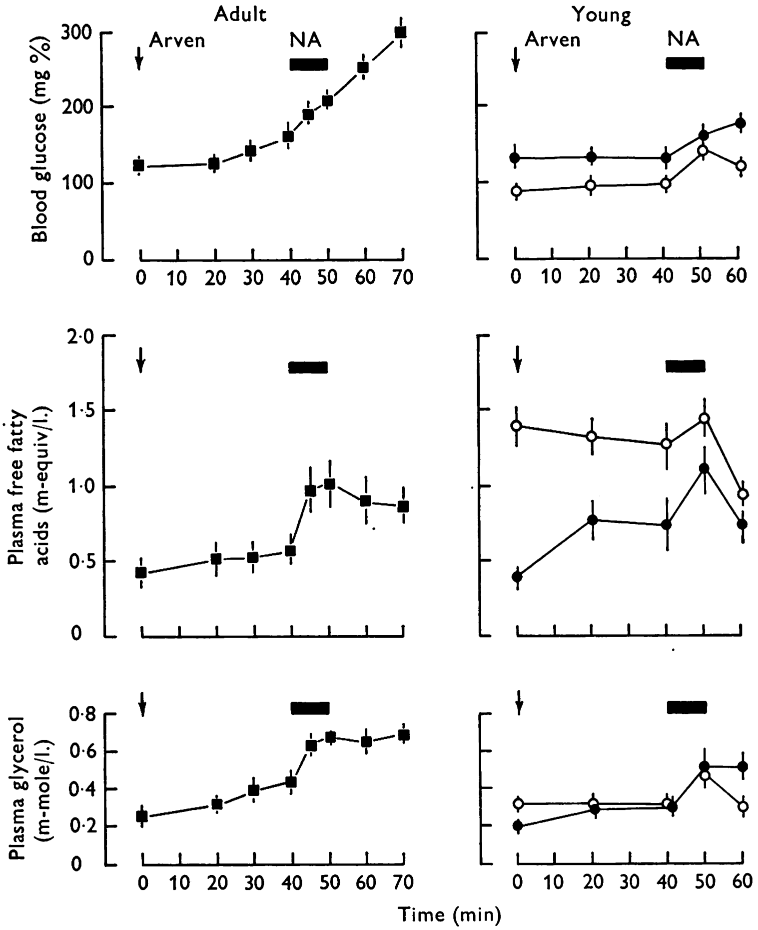

Fig. 1. The mean arterial concentrations of blood glucose, plasma, free fatty acids and plasma glycerol in fed adult (■) and fed (●) or starved (○) 3-month-old rabbits before, during and after infusions of noradrenaline (4 μg/kg.min I.V.). Anticoagulation was achieved with Arven (0·67 u./kg. I.V.) after the initial blood samples were taken. The vertical lines in this and the subsequent figure represent the S.E. of mean.

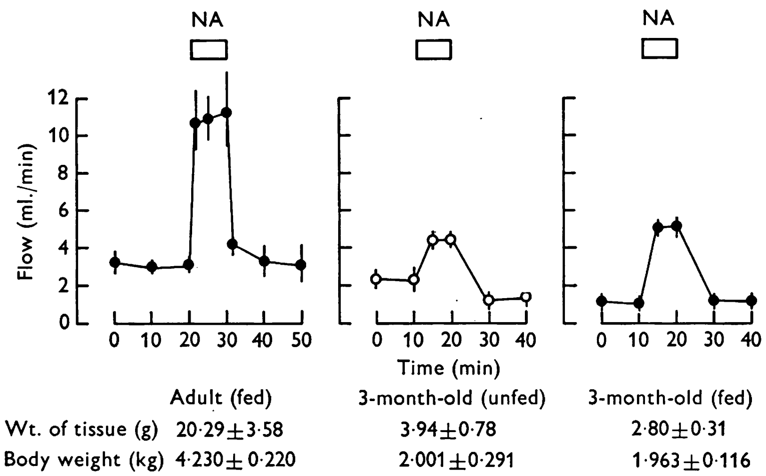

Fig. 2. The rate of blood flow from the lateral vein of cervical adipose tissue in fed adult and fed or starved 3-month-old rabbits, before, during and after infusion of noradrenaline (NA) (4 μg/kg.min I.V.) for 10 min. The mean ($\pm$S.E. of mean) weight of the lateral and upper half of the posterior lobe for each group is given as is the body weight.

TABLE 1. The mean ($\pm$S.E. of mean) arterio-venous differences of blood glucose, plasma free fatty acids and plasma glycerol across the cervical adipose tissue of fed or starved three-month-old rabbits and fed adult rabbits. In each group the numbers of observations made before, during and after infusions of noradrenaline (4 μg/kg. min.I.V. for 10 min) are given

		Arterio-venous differences		
	No. of observations	Blood glucose (mg/100 ml.)	Plasma free fatty acids (m-equiv/l.)	Plasma glycerol (m-mole/l.)
Five 3-month-old rabbits (fed)				
Initial	10	−0·6 ± 2·1	+0·18 ± 0·06	+0·12 ± 0·03
During noradrenaline infusion	7	−2·0 ± 2·4	+0·35 ± 0·06	+0·11 ± 0·02
Post-infusion	9	−2·9 ± 3·3	+0·47 ± 0·07	+0·30 ± 0·05
Five 3-month-old rabbits (starved)				
Initial	13	−1·7 ± 2·3	+0·37 ± 0·03	+0·16 ± 0·02
During noradrenaline infusion	5	−1·4 ± 3·1	+0·24 ± 0·09	+0·14 ± 0·03
Post-infusion	6	+2·8 ± 1·4	+0·42 ± 0·08	+0·23 ± 0·04
Six 7-month-old rabbits (fed)				
Initial	15	−3·3 ± 1·6	+0·18 ± 0·05	+0·14 ± 0·03
During noradrenaline infusion	9	+1·5 ± 2·8	+0·17 ± 0·04	+0·11 ± 0·02
Post-infusion	9	−4·1 ± 5·3	+0·18 ± 0·07	+0·13 ± 0·02

difference of fatty acids persisted after the infusion had stopped. In contrast the arterio-venous difference of fatty acids in the unfed young rabbits fell when blood flow increased and returned to resting levels after the infusion. Nevertheless, there was an increase in fatty acid release from 0·23 to 0·28 μequiv/g tissue.min and glycerol from 0·10 to 0·16 μmole/g tissue.min. In the adult rabbits the arterio-venous difference was unchanged during the noradrenaline infusion but as the flow did increase the net release of fatty acids from cervical adipose tissue increased threefold from 0·03 to 0·09 μequiv/g tissue.min and glycerol from 0·02 to 0·06 μmole/g tissue.min. As in the young rabbits the high rate of release of fatty acids and glycerol was accompanied by a sharp rise in arterial concentrations of these products. The arterial concentrations of glucose tended to rise in response to noradrenaline but the arterio-venous difference of glucose was very variable.

In both the young and adult rabbits there was no change in colonic temperature in response to noradrenaline infusion. Of the five adult rabbits studied only two had a small and unpredictable rise in temperature in cervical adipose tissue (0·3 and 0·5° C respectively), whilst all five animals had a threefold increase in blood and fatty acid release in response to the infusion.

Noradrenaline 4μg/kg.min caused a sharp rise in blood pressure and fall in heart rate within seconds of commencing the infusion, but these changes gradually returned towards the initial levels towards the end of a 10 min infusion.

DISCUSSION

In the new-born rabbit the function of cervical adipose tissue is to generate extra heat in response to cold exposure by oxidizing the fat it stores. The tissue is well equipped for this function, it contains all the necessary enzymes for the oxidation of fatty acids and it has a high rate of blood flow (on average 1·0 ml./g tissue.min) and oxygen consumption. At birth the fat stored in cervical adipose tissue is preferentially retained during starvation. At this stage cervical adipose tissue is functionally distinct from the more familiar white adipose tissue which readily releases fatty acids for metabolism elsewhere (Hull & Segall, 1966; Heim & Kellermayer, 1967). By 1 week of age the function of cervical adipose tissue has begun to change. First, the tissue releases the fatty acids it stores into the circulation and the net release increases in response to starvation. Secondly, thermogenic capacity of the tissue has begun to fall (Hardman & Hull, 1971).

In the present experiments, noradrenaline which is a powerful stimulus to non-shivering heat production in the new-born rabbit (Hull & Segall, 1965) did not cause a rise in colonic temperature. This finding, together with the fact that a consistent and reproducible rise did not occur in the temperature of cervical adipose tissue, leads to the conclusion that the cervical adipose tissue in adult rabbits reared under animal house conditions has little or no capacity to generate extra heat.

The resting rate of blood flow through cervical adipose tissue in 3-month-old rabbits was on average 0·45 ml./g tissue.min compared with 1·00 ml./g tissue.min in new-born rabbits. In the adult rabbits the mean rate of blood flow through cervical adipose tissue was even lower at 0·16 ml./g tissue.min but this is nevertheless at least ten times higher than that reported for white adipose tissue in rabbits (Lewis & Mathews, 1968). The arterio-venous difference of fatty acids across cervical adipose tissue was similar to that across white adipose tissue and therefore in view of the higher rate of blood flow the net release of fatty acids was considerably greater. As might have been anticipated the rate of release of fatty acids from cervical adipose tissue increased still further in response to starvation, partly as a result of an increase in the rate of blood flow and partly as a result of an increase in the arterio-venous difference of fatty acids.

Noradrenaline stimulated a large increase in blood flow and fatty acid release from the cervical adipose tissue in 1-week-old rabbits (Hardman & Hull, 1970). It could be argued that the increase in blood flow through cervical adipose tissue in response to noradrenaline in new-born rabbits is due to the increase in the tissues' oxygen requirements for thermogenesis. However, similar increases in the flow occurred in young and adult rabbits when thermogenesis did not occur. It is possible that noradrenaline has a direct vasodilator action on the blood vessels of cervical adipose tissue, or that an increased rate of fatty acid release in some way leads to an increase in blood flow.

The effect of noradrenaline on cervical adipose tissue is in direct contrast to its effect on white adipose tissue, where it causes vasoconstriction and a decrease in fatty acid release (Lewis & Mathews, 1968). The sharp rise is the circulating concentration of free fatty acids which occurs with noradrenaline infusion presumably due, in part at least, to the high rate of fatty acid release from cervical adipose tissue.

In conclusion it has been shown that cervical adipose tissue has a high resting blood flow and that the net release of fatty acids increases in response to starvation or noradrenaline infusion. In the adult rabbit this tissue obviously plays a more active role in energy homoeostasis than does white adipose tissue. Its function, however, is different from that of brown adipose tissue in the new-born rabbit.

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